B1 cont.

through 948 of the cDNA sequence as shown in FIG. 1; spanning nucleotides 3108 and 3614 of the cDNA of FIG.6) and it recognizes three fragments on EcoRI-digested genomic and BAC DNA (approximately sized at 1.5kb, 4.3kb, and 9kb).

Please replace the paragraph beginning at page 8, line 14, with the following rewritten paragraph:

B2

Figure 7. Sequence of human CLASP-3 exons and introns, and potential promoter. **A.** Sequence of human CLASP-3 exons and intron borders (SEQ ID NOS:82-97). Stretches of noncontiguous genomic sequence from the Human Genome Project (GENBANK entry gi9212047) were aligned using the human CLASP-3 cDNA as a template and Sequencher sequence analysis software (Gene Codes Corp). 15 exons representing approximately the 5' 10% of the human CLASP-3 cDNA sequence are presented in predicted 5' to 3' order. Exon sequences are underlined and are flanked by intron sequence. This exon/intron map could only have been produced having the isolated human CLASP-3 cDNA. Nucleotide numbers for each exon and flanking intron sequences are indicated and represent the annotation found in Genbank entry gi9212047. Note that these sequences and numbers are with respect to the reverse complement (anti-parallel) of the nucleotides in Genbank entry gi9212047. **B**. Genomic nucleotide sequence (SEQ ID NO:98) upstream of the human CLASP-3 5' terminus, which represents the putative promoter region for human CLASP-3. The first exon of the CLASP-3 cDNA is underlined. Nucleotides 58000 to 60348 of the reverse complement of gi9212047 are shown.

Please replace the paragraph beginning at page 8, line 29, with the following rewritten paragraph:

B3

Figure 8. Amino acid alignment and comparison between the human (h) CLASP family members (SEQ ID NOS:99-104). Amino acid sequences were aligned using ClustalW. The alignment is presented in order of their greatest pairwise similarity scores. Single letter amino acid abbreviations are used. Asterisks indicate complete

identity, while colons and periods indicate sequence similarity among CLASP family members. Dashes indicate gaps inserted in the amino acid sequence to facilitate alignment. Labeled boxes are domains with similarity to known protein motifs; unlabeled boxes represent regions of similarity between all CLASPs and may represent CLASP-specific domains.

B3 cont.

Please replace the paragraph beginning at page 8, line 29, with the following rewritten paragraph:

B4

The phrase "sequence similarity" in the context of two nucleic acids or polypeptides, refers to two or more sequences that are identical or in the case of amino acids, have homologous amino acid substitutions at either 50%, often at least 60%, often at least 70%, preferably at least 80%, most preferably at least 90% or at least 95% of the indicated positions.

Please replace the paragraph beginning at page 8, line 29, with the following rewritten paragraph:

B5

Similarly, products of an immune response in either a model organism (*e.g.*, mouse) or a human patient can also be detected by various methods that are well known to those of ordinary skill in the art. For example, (1) the production of antibodies in response to vaccination can be readily detected by standard methods currently used in clinical laboratories, *e.g.*, an ELISA; (2) the migration of immune cells to sites of inflammation can be detected by scratching the surface of skin and placing a sterile container to capture the migrating cells over scratch site (Peters *et al.*, 1988, Blood 72: 1310-5); (3) the proliferation of peripheral blood mononuclear cells in response to mitogens or mixed lymphocyte reaction can be measured using 3H-thymidine; (4) the phagocitic capacity of granulocytes, macrophages, and other phagocytes in PBMCs can be measured by placing PMBCs in wells together with labeled particles (Peters *et al.*, 1988); and (5) the differentiation of immune system cells can be measured by labeling

B5 cont.

PBMCs with antibodies to CD molecules such as CD4 and CD8 and measuring the fraction of the PBMCs expressing these markers.

Please replace the paragraph beginning at page 17, line 6, with the following rewritten paragraph:

Similarly, products of an immune response in either a model organism (*e.g.*, mouse) or a human patient can also be detected by various methods that are well known to those of ordinary skill in the art. For example, (1) the production of antibodies in response to vaccination can be readily detected by standard methods currently used in clinical laboratories, *e.g.*, an ELISA; (2) the migration of immune cells to sites of inflammation can be detected by scratching the surface of skin and placing a sterile container to capture the migrating cells over scratch site (Peters *et al.*, 1988, Blood 72: 1310-5); (3) the proliferation of peripheral blood mononuclear cells in response to mitogens or mixed lymphocyte reaction can be measured using 3H-thymidine; (4) the phagocytic capacity of granulocytes, macrophages, and other phagocytes in PBMCs can be measured by placing PMBCs in wells together with labeled particles (Peters *et al.*, 1988); and (5) the [differentiation] differentiatian of immune system cells can be measured by labeling PBMCs with antibodies to CD molecules such as CD4 and CD8 and measuring the fraction of the PBMCs expressing these markers.

B6

Please replace the paragraph beginning at page 22, line 32, with the following rewritten paragraph:

B7

CLASP-3 predicted amino acid sequence was analyzed using the PHDhtm analysis software for prediction of transmembrane helices (Rost, B., et al., 1996, Prot. Science 7: 1704-1718). Using the PPHDhtm analysis software, it was determined that a transmembrane domain is located from nucleotides 2417-2473 (as shown in FIG. 1; nucleotides 5080 to 5136 as shown in FIG. 6). Other potential transmembrane domains are located in the amino terminal 1693 amino acids as shown in FIG. 6.

Please replace the paragraph beginning at page 31, line 3, with the following rewritten paragraph:

B8

Standard assays can be used for detecting CLASP protein interaction with cytoskeletal proteins. These assays include co-sedimentation assays, far western blot analysis (Ohba, T., 1998, Anal. Biochem. 262: 185-192), surface pasman resonance, F-actin staining with phalloidin in CLASP-transfected lymphocytes (*e.g.*, Small, J. *et al.* 1999, Microsc. Res. Tech. 4: 3-17), and immunocytol analysis of subcellular distribution of focal adhesion proteins (such as paxillin, tensin, vinculin, talin, and FAK in CLASP-transfected lymphocytes; see, *e.g.*, Ridyard, M.S., 1998, Biochem. Cell Biol. 76: 45-58).

Please replace the paragraph beginning at page 32, line 19, with the following rewritten paragraph:

B9

As is illustrated in FIG. 3, CLASP-3 is a member of a superfamily of immune-cell associated proteins with similar motifs (e.g., CLASP-1, 2/6, 3, 4, 5, 7). CLASP-1 is described in WO 00/20434. CLASP-1 uniquely among the known CLASPs contains SH3 binding domain motifs. CLASP-2 is described in WO 00/61747. CLASP-2 polypeptides have no adaptor binding sites or SH3 binding domains found in CLASP-1. Other CLASP family members are described in Application Nos. 09/736,969; 09/736,960; 09/736,968 (all filed December 13, 2000), 60/240,508, 60/240,503, 60/240,539, and 60/240,543 (all filed October 13, 2000). The aforementioned publications and applications are all incorporated by reference herein in its entirely for all purposes.

Please replace the paragraph beginning at page 37, line 14, with the following rewritten paragraph:

B10

In some embodiments, the CLASP-3 polynucleotides of the invention are substantially identical to SEQ ID NO:1 or to a fragment thereof.

Please replace the paragraph beginning at page 38, line 9, with the following rewritten paragraph:

"Stringent hybridization conditions" are conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acid, but not to other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at Tm, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For high stringency hybridization, a positive signal is at least two times background, preferably 10 times background hybridization. Exemplary high stringency or stringent hybridization conditions include: 50% formamide, 5x SSC and 1% SDS incubated at 42° C or 5x SSC and 1% SDS incubated at 65° C, with a wash in 0.2x SSC and 0.1% SDS at 65° C. In a specific embodiment, a nucleic acid which is hybridizable to a CLASP-3 nucleic acid under the following conditions of high stringency is provided: Prehybridization of filters containing DNA is carried out for 8 h to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml

B''

denatured salmon sperm DNA. Filters are hybridized for 8-16 h at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 65°C for 15-30 h in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.2X SSC and 0.1% at 50°C for 15-30 min before autoradiography.

B11 cont.

Please replace the paragraph beginning at page 42, line 20, with the following rewritten paragraph:

A variety of uses of the CLASP promoter sequence provided herein will be apparent to one of skill reviewing this disclosure. In an embodiment, reporter genes are operably linked to CLASP upstream sequences containing promoter elements. The resulting vectors have numerous uses, including identification of cis and trans transcriptional regulatory factors in vivo and for screening of agents capable of modulating (e.g., activating or inhibiting) CLASP expression (e.g., drug screening). In an embodiment, for example, a modulator of CLASP expression can be identified by detecting the effect of the modulator on expression of a reporter gene whose expression is regulated, in whole or part, by a naturally occurring CLASP regulatory element (e.g., promoter or enhancer). A number of reporters may be used (e.g., firefly luciferase, β-glucuronidase, β-galactosidase, chloramphenicol acetyl transferase, SEAP, GFP). In a related embodiment, a CLASP coding sequence is used in place of a reporter and changes in CLASP protein expression (or activity) is detected using the methods disclosed herein. In a related embodiment, the ability of a test compound to bind to a CLASP gene regulatory sequence is assayed.

B12

Please replace the paragraph beginning at page 45, line 13, with the following rewritten paragraph:

Methods of selecting nucleic acid probe sequences for use in nucleic acid hybridization are discussed in Sambrook *et al.*, supra. In some formats, at least one of the

B13

target and probe is immobilized. The immobilized nucleic acid can be DNA, RNA, or another oligo- or poly-nucleotide, and can comprise natural or non-naturally occurring nucleotides, nucleotide analogs, or backbones. Such assays can be in any of several formats including: Southern, Northern, dot and slot blots, high-density polynucleotide or oligonucleotide arrays (*e.g.*, GeneChips™ Affymetrix), dip sticks, pins, chips, or beads. All of these techniques are well known in the art and are the basis of many commercially available diagnostic kits. Hybridization techniques are generally described in Hames *et al.*, ed., 1985, Nucleic Acid Hybridization, A Practical Approach IRL Press; Gall and Pardue, 1969, Proc. Natl. Acad. Sci. U.S.A., 63: 378-383; and John *et al.*, 1969, Nature, 223: 582-587.

B13 cont.

Please replace the paragraph beginning at page 51, line 1, with the following rewritten paragraph:

In another embodiment, hCLASP-3-based diagnostics involve screening assays for identifying disorders of cells of hematopoietic lineage. hCLASP-3 is expressed in human T cells, B cell lines and in myelomonocytic cells but not monocytic or myelocytic cells. In the French-American-British (FAB) classification of acute myelogenous leukemias, M4 (acute myelomonocytic leukemia) has a subset (M4eo) that is associated with excellent prognosis (Cotran, et al. Robbins Pathologic Basis of Disease, 6th edition, Saunders, 1999). The use of hCLASP-3 may help to further subdivide M4 and permit a better forecast of prognosis. Precise identification of hematopoietic cell types is vital to guide chemotherapy and radiation therapy of patients with leukemia and lymphoma (Fauci et al., (eds.), 1998, Harrison's Principles of Internal Medicine, 14th Ed., McGraw Hill, pp. 695-712). hCLASP-3 expression differences can be detected by using FACS, immunofluorescence, immunoperoxidase staining, RT-PCR, in situ hybridization or RNA blot analysis (Sambrook, Fritsch and Maniatas, 1989, Molecular Cloning, 2nd Ed., Cold Spring Harbor Lab. Press; Ward MS, 1999, Pathology 31(4): 382-92).

B14

Please replace the paragraph beginning at page 53, line 16, with the following rewritten paragraph:

It will be appreciated that the CLASP-3 polynucleotides and oligonucleotides of the invention can be made using nonstandard bases (*e.g.*, other than adenine, cytidine, guanine, thymine, and uridine) or nonstandard backbone structures to provides desirable properties (*e.g.*, increased nuclease-resistance, tighter-binding, stability or a desired TM). Techniques for rendering oligonucleotides nuclease-resistant include those described in PCT publication WO 94/12633. A wide variety of useful modified oligonucleotides may be produced, including oligonucleotides having a peptide-nucleic acid (PNA) backbone (Nielsen *et al.*, 1991, Science 254: 1497) or incorporating 2'-O-methyl ribonucleotides, phosphorothioate nucleotides, methyl phosphonate nucleotides, phosphotriester nucleotides, phosphorothioate nucleotides, phosphoramidates. Still other useful oligonucleotides may contain alkyl and halogen-substituted sugar moieties comprising one of the following at the 2' position: OH, SH, SCH3, F, OCN, OCH3OCH3, OCH3O(CH2)nCH3, O(CH2)nNH2 or O(CH2)nCH3, where n is from 1 to about 10; C1 to C10 lower alkyl, substituted lower alkyl, alkaryl or aralkyl; Cl; Br; CN; CF3; OCF3; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; SOCH3 ; SO2CH3; ONO2; NO2; N3; NH2; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a cholesteryl group; a folate group; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an oligonucleotide and other substituents having similar properties. Folate, cholesterol or other groups that facilitate oligonucleotide uptake, such as lipid analogs, may be conjugated directly or via a linker at the 2' position of any nucleoside or at the 3' or 5' position of the 3'-terminal or 5'-terminal nucleoside, respectively. One or more such conjugates may be used. Oligonucleotides may also have sugar mimetics such as cyclobutyls in place of the pentofuranosyl group. Other embodiments may include at least one modified base form or "universal base" such as

B5

inosine, or inclusion of other nonstandard bases such as queosine and wybutosine as well as acetyl-, methyl-, thio- and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases. The antisense oligonucleotide can comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5β-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

B15

cont.

Please replace the paragraph beginning at page 57, line 1, with the following rewritten paragraph:

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site can be evaluated for predicted structural features such as secondary structure that can render the oligo-nucleotide sequence unsuitable. The suitability of candidate targets can also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

B16

Please replace the paragraph beginning at page 67, line 7, with the following rewritten paragraph:

The present invention provides new antibacterial agents. Certain CLASP-3 DNA sequences were difficult to clone and subclone (see Example 1). Bacteria harboring certain pieces of CLASP cDNA products were unable to be isolated, indicating that introduction of CLASP sequences compromised bacterial viability. There can be at least two possible reasons why the CLASP cDNA were unable to be cloned, which can reflect a variation of the well-established Modification and Restriction systems found in bacteria (reviewed in Wilson and Murray. (1991) Annu. Rev. Genet. 25:585-627; Bickle and Kruger (1993) Microbiol. Rev. 57:29-67). This well-described system is used by bacteria to prevent deleterious effects caused by the introduction of foreign DNA. Bacteria can recognize foreign DNA since it does not have the same modifications (e.g. methylation) as the native DNA. After recognition, the bacteria then digest and eliminate the foreign DNA (restriction). In the first scenario, the CLASP cDNA can be recognized as foreign DNA, and digested and eliminated as in the Modification and Restriction system. However, this would be unique for CLASP cDNA since the bacteria used for cloning cDNA are compromised in the Modification and Restriction system, which makes cloning of cDNA into bacteria a practice common in the art. If this is the case, the bacterial apparatus that specifically recognizes or eliminates CLASP cDNA can provide a novel target to develop antimicrobial agents. The CLASP DNA sequence would be useful in targeting the apparatus as well as an entry point for designing screens to identify potential targets. The second possibility is that CLASP cDNA behaves as an antimicrobial agent (i.e., antibiotic), and prevents bacterial growth. This, in effect, would create a new type of antibiotic mediated by the presence of foreign DNA (i.e. CLASP cDNA). In the case for the CLASP cDNA, the bacteria can recognize the DNA but instead of digesting and eliminating the DNA, the CLASP cDNA can cause a variation of the restriction and prevent the bacteria from growing, imposing a bactericidal effect upon the bacteria.

Please replace the paragraph beginning at page 74, line 8, with the following rewritten paragraph:

In cases where plant expression vectors are used, the expression of the CLASP-3 coding sequence can be driven by any of a number of promoters. For example, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV (Brisson et al., 1984, Nature 310: 511-514), or the coat protein promoter of TMV (Takamatsu et al., 1987, EMBO J. 6: 307-311) can be used; alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi et al., 1984, EMBO J. 3: 1671-1680; Broglie et al., 1984, Science 224: 838-843); or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B (Gurley et al., 1986, Mol. Cell. Biol. 6: 559-565) can be used. These constructs can be introduced into plant cells using Ti plasmids, Ri plasmids, plant virus vectors, direct DNA transformation, microinjection, electroporation, and the like. (Weissbach & Weissbach, 1988, Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp. 421-463; and Grierson & Corey, 1988, Plant Molecular Biology, 2d Ed., Blackie, London, Ch. 7-9.).

B 18

Please replace the paragraph beginning at page 76, line 3, with the following rewritten paragraph:

Host cells transformed with nucleotide sequences encoding CLASP-3 may be cultured under conditions suitable for the expression and recovery of the soluble protein from cell culture. The protein produced by a transformed cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode CLASP-3 may be designed to contain signal sequences which direct secretion of CLASP-3 through a prokaryotic or eukaryotic cell membrane. Other constructions may be used to join sequences encoding CLASP-3 to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides

B 19

B19 cont.

such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin.

Please replace the paragraph beginning at page 77, line 14, with the following rewritten paragraph:

In an alternate embodiment of the invention, the coding sequence of CLASP-3 could be synthesized in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers et al., 1980, Nuc. Acids Res. Symp. Ser. 7: 215-233; Crea and Horn, 180, Nuc. Acids Res. 9(10): 2331; Matteucci and Caruthers, 1980, Tetrahedron Letter 21: 719; and Chow and Kempe, 1981, Nuc. Acids Res. 9(12): 2807-2817.)

B20

Alternatively, the protein itself could be produced using chemical methods to synthesize a CLASP-3 amino acid sequence in whole or in part. For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography. (See Creighton, 1983, Proteins Structures And Molecular Principles, W.H. Freeman and Co., N.Y. pp. 50-60). The composition of the synthetic polypeptides can be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; see Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman and Co., N.Y., pp. 34-49).

Please replace the paragraph beginning at page 83, line 29, with the following rewritten paragraph:

B21

Various procedures known in the art can be used for the production of antibodies to epitopes of the natural and recombinantly produced CLASP-3 protein. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, human or humanized, IgG, IgM, IgA, IgD or IgE, a complementarity determining region, Fab fragments, F(ab')2 and fragments produced by an Fab expression library as well as anti-idiotypic antibodies. Antibodies which compete for CLASP-3 binding are especially preferred for diagnostics and therapeutics.

Please replace the paragraph beginning at page 84, line 14, with the following rewritten paragraph:

For the production of antibodies, various host animals can be immunized by injection with the recombinant or naturally purified CLASP-3 protein, fusion protein or peptides, including but not limited to goats, rabbits, mice, rats, hamsters, and the like Various adjuvants can be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacilli Calmette-Guerin) and Corynebacterium parvum.

B22

Please replace the paragraph beginning at page 84, line 23, with the following rewritten paragraph:

Monoclonal antibodies to CLASP-3 can be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique originally described by Kohler and Milstein, (Nature, 1975, 256: 495-497), the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today, 4: 72; Cote et al., 1983, Proc. Natl. Acad. Sci. U.S.A., 80: 2026-2030) and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A., 81: 6851-6855; Neuberger et al., 1984, Nature, 312: 604-608; Takeda et al., 1985, Nature, 314: 452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce CLASP-3 -specific single chain antibodies. In

B23

B23 cont.

some embodiments, phage display technology is used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (see, e.g., McCafferty et al., Nature 348: 552-554 (1990); Marks et al., Biotechnology 10: 779-783 (1992)).

Please replace the paragraph beginning at page 87, line 18, with the following rewritten paragraph:

B24

CLASP-3 activities include, for example, the CLASP-3 polypeptide involvement in signal transduction (e.g., leading to T cell activation). Compounds or agents that modulate the interaction of a CLASP-3 polypeptide and a target molecule, modulate CLASP-3 nucleic acid expression, or modulate CLASP-3 polypeptide activity are all contemplated by the methods of the present invention.

Please replace the paragraph beginning at page 88, line 27, with the following rewritten paragraph:

B25

Typically, the assays are recombinant cell based or cell-free assay. These assays can include the steps of combining a cell expressing a CLASP-3 polypeptide or a binding fragment thereof, a CLASP-3 target molecule (e.g., a CLASP-3 ligand) and a test compound, under conditions where but for the presence of the candidate compound, the CLASP-3 polypeptide or biologically active portion thereof binds to the target molecule. Detecting complex formation between the CLASP-3 polypeptide or the binding fragment thereof, the CLASP-3 target molecule and a test compound detecting the formation of a complex which includes the CLASP-3 polypeptide and the target molecule can be accomplished. Detection of complex formation can include direct quantitation of the complex by, for example, measuring inductive effects, such as T cell activation, of the CLASP-3 polypeptide. A significant change, such as a decrease, in the interaction of the CLASP-3 and target molecule (e.g., in the formation of a complex between the CLASP-3 and the target molecule) in the presence of a candidate compound (relative to what is

detected in the absence of the candidate compound) is indicative of a modulation of the interaction between the CLASP-3 polypeptide and the target molecule. Modulation of the formation of complexes between the CLASP-3 polypeptide and the target molecule can be quantitated using, for example, an immunoassay. To perform cell free drug screening assays, it is desirable to immobilize either CLASP-3 or its target molecule to facilitate separation of complexes from uncomplexed forms of one or both of the polypeptides, as well as to accommodate automation of the assay. CLASP-3 binding to a target molecule, in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and microcentrifuge tubes.

B25 cont.

Please replace the paragraph beginning at page 97, line 27, with the following rewritten paragraph:

B26

Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds in a short period. See, e.g., Fodor et al., 1991, Science 251: 767-773, and other descriptions of chemical diversity libraries, which describe means for testing of binding affinity by a plurality of compounds.

Please replace the paragraph beginning at page 102, line 32, with the following rewritten paragraph:

B27

In one embodiment, a standard animal model for graft versus host rejection is ectopic heart transplantation (Fulmer et al., 1963, Am. J. Anat. 113: 273-281). This method involves using BALB/C mice (either sex, and range from 1–9 months) for transplanting cardiac tissue into a surgically-created pocket on the dorsum for both ears made by slitting the skin over the auricular artery at the base of the ear. Small curved forceps are forced into the slit, bluntly dissecting between the skin and the cartilage plate. Donor tissue is eased into the base of the pocket near the distal edge of the ear. The

B27 cont.

auricular artery is used to seal off the opening of the pocket. Within 10 to 14 days pulsatile activity of the transplant should be observed. Gross appearance of the graft, patterns of vacuolar supply to the graft area and pulsatile activity can be easily observed utilizing transilluminated light during the first three weeks post-transplantation. Follow-up can continue for several months.

Please replace the paragraph beginning at page 103, line 19, with the following rewritten paragraph:

B28

Once a titer of the potency of collagen type II (CII) is obtained therapeutics can be tested. In one embodiment, three mice will be immunized with three different concentrations of CII 50, 200, and 400 μg per animal (Nabozny *et al.*, 1996, J. Exp. Med., 183: 27-37). To induce CIA, animals can be immunized with an appropriate concentration of CII, determined as described above. One half of a 1:1 ratio of antigen:CFA can be injected at the base of the tail and the remainder equally divided in each hind footpad. Mice can be carefully monitored every day for the onset and progression of CIA throughout the experiment until its termination 12 weeks post-immunization with CII. The pieces of heart transplanted can be approximately 3 X 3 mm in size. The severity of arthritis can be assessed following standard procedures known to one of skill in the art.

Please replace the line beginning at page 104, line 31, with the following line:

B29

(1) ^{3}H-thymidine incorporation

Please replace the paragraph beginning at page 109, line 24, with the following rewritten paragraph:

B30

To obtain additional 5' CLASP-3 sequence, portions of the cDNA and genomic sequence from a BAC (Bacterial Artificial Chromosome) genomic library were compared to the NCBI database by BLAST. A genomic clone (Genbank identifier:

giAL138847 comprising random, shotgun genomic sequence was identified. Using TFASTX (Pearson and Lipman, Proc. Natl. Acad. Sci U.S.A. (1988) 85:2444-2448), the amino-terminal sequence of human CLASP-4 was compared to 6 frame translation of giAL138847 . Areas of giAL138847 that encoded amino acids with high similarity to CLASP4 amino acid sequence were used to design CLASP-3-specific oligonucleotides for RTPCR (reverse transcriptase polymerase chain reaction according to manufacturers instructions: Reverse transcriptase Gibco/BRL, Taq Polymerase from Sigma). Using sense oligonucleotides HC3gS5 (nucleotides 275-279 of FIG. 6) and HC3gS6 (nucleotides 351-374) and antisense oligonucleotide HC3AS7 (reverse complement of nucleotides 3074-3093 of FIG. 6) an RTPCR product of approximately 3.0 kb was generated, sequenced (dideoxynucleotide termination sequencing, Beckman Coulter CEQ2000) and shown to be additional human CLASP-3 5' sequence. Further complicating the cloning full-length CLASP cDNA products was the difficulty to clone (and subclone) certain CLASP cDNA products. Standard isolation of some of the CLASP cDNAs from a pure phage population following screening of commercially available cDNA libraries ("ZAP-out" procedure, Stratagene) resulted in no bacterial colonies. Similarly, certain RT-PCR products could not be cloned into standard plasmid vectors. No colonies were isolated by cloning these fragments into vectors lacking promoters, reverse orientations, low copy vectors, or by growth at altered temperatures or levels of antibiotic for plasmid selection (examples: CLASP-7 - HC7gS6 to HC7gAS1 and HC7gS3 to HC7AS14; CLASP-4 – C4P2 to hC4ASTM and C4P2 to HC4AS3'; CLASP-1 – hC1S5' to hC1AS3'Kpn and C1S7 to hC1AS3'Kpn; see Primer Table below). One possibility is that sequences contained within certain regions of CLASP cDNAs are bactericidal and therefore not amenable to cloning. To circumvent these problems direct sequencing of RT-PCR products was performed.

Please replace the paragraph beginning at page 106, line 4, with the following rewritten paragraph:

Cell supernatants harvested after cell stimulation for 16-48 hrs are stored at -80°C until assayed or directly tested for cytokine production. Multiple cytokine assays can be performed on each sample. IL-2, IL-3, IFN-γ and other cytokine ELISA Assays are available for mouse, rat, and human (Endogen, Inc. and BioSource). Cytokine production is measured using a standard two-antibody sandwich ELISA protocol as described by the manufacturer. The presence of horseradish peroxidase is detected with 3, 3'5, 5' tertamethyl benziidine (TMB) substrate and the reaction is stopped with sulfuric acid. The absorbency at 450 nm is measured using a microplate reader. Any statistically significant increase or decrease in color intensity of CLASP-3-treated sample, as compared to control sample (no treatment), suggest an effect of CLASP-3 on biological function.

B31

Please replace the lined at page 107, line 30, with the following line:

B32

(M) Structure/Function Assays: Homotypic and/or Heterotypic, Calcium-dependent Cell Adhesion

Please replace the paragraph beginning at page 109, line 24, with the following rewritten paragraph:

B33

To obtain additional 5' CLASP-3 sequence, portions of the cDNA and genomic sequence from a BAC (Bacterial Artificial Chromosome) genomic library were compared to the NCBI database by BLAST. A genomic clone (Genbank identifier: giAL138847 comprising random, shotgun genomic sequence was identified. Using TFASTX (Pearson and Lipman, PNAS (1988) 85:2444-2448), the amino-terminal sequence of human CLASP-3 was compared to 6 frame translation of giAL138847 . Areas of giAL138847 that encoded amino acids with high similarity to CLASP-3 amino acid sequence were used to design CLASP-3-specific oligonucleotides for RTPCR (reverse transcriptase polymerase chain reaction according to manufacturers instructions:

Reverse transcriptase Gibco/BRL, Taq Polymerase from Sigma). Using sense oligonucleotides HC3gS5 (nucleotides 275-279 of FIG. 6) and HC3gS6 (nucleotides 351-374) and antisense oligonucleotide HC3AS7 (reverse complement of nucleotides 3074-3093 of FIG. 6) an RTPCR product of approximately 3.0 kb was generated, sequenced (dideoxynucleotide termination sequencing, Beckman Coulter CEQ2000) and shown to be additional human CLASP-3 5' sequence. Further complicating the cloning full-length CLASP cDNA products was the difficulty to clone (and subclone) certain CLASP cDNA products. Standard isolation of some of the CLASP cDNAs from a pure phage population following screening of commercially available cDNA libraries ("ZAP-out" procedure, Stratagene) resulted in no bacterial colonies. Similarly, certain RT-PCR products could not be cloned into standard plasmid vectors. No colonies were isolated by cloning these fragments into vectors lacking promoters, reverse orientations, low copy vectors, or by growth at altered temperatures or levels of antibiotic for plasmid selection (examples: CLASP-7 - HC7gS6 to HC7gAS1 and HC7gS3 to HC7AS14; CLASP-4 – C4P2 to hC4ASTM and C4P2 to HC4AS3'; CLASP-1 – hC1S5' to hC1AS3'Kpn and C1S7 to hC1AS3'Kpn; see Primer Table below). One possibility is that sequences contained within certain regions of CLASP cDNAs are bacteriacidal and therefore not amenable to cloning. To circumvent these problems direct sequencing of RT-PCR products was performed.

B33 cont.

Please replace the paragraph beginning at page 111, line 15, with the following rewritten paragraph:

B34

RACE was carried out using Invitrogen's Generacer kit according to manufacturers specifications using polyA selected mRNA from 9D10 B cell tissue culture line. The sequence of the oligonucleotides presented is the reverse complement (i.e. antisense) of the CLASP-3 cDNA at the indicated position based upon numbering in FIG. 6.

Please replace the paragraph beginning at page 111, line 25, with the following rewritten paragraph:

Expression of human CLASP-3 was evaluated in different cell lines using DNA transfection of tagged fusion protein constructs. Two standard methods for DNA transfection were used, DNA precipitation by calcium phosphate (Graham, F. and van der Eb, A. and Gorman, C. et al.,) and electroporation (Potter, H.). Two different expression systems were used to analyze HC3 expression in mouse and human lymphocytic cells and in human 293 cells, RFP (DsRED) and human IgG (Fc specific fragment). Both expression systems were designed to control for protein folding of HC3 by expressing the tagged protein C-terminal to HC3.

B35

Please replace the paragraph beginning at page 112, line 6, with the following rewritten paragraph:

Jurkat E6 human T cells, (ATCC TIB-152), were maintained and tested in complete IMDM (IMDM medium supplemented with 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate (Gibco BRL), 50 μM beta mercaptoethanol (Sigma), and 10% fetal calf serum (Gemini Bio-Products)). Human embryonic kidney cells, 293, were maintained and tested in complete DMEM (DMEM medium supplemented with 2 mM glutamine, 10 mM HEPES, 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% fetal calf serum). CH27 mouse B cell lymphoma and 2B4 mouse T cell hybrid were maintained and tested in complete RPMI (RPMI 1640 medium supplemented with 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 10 mM HEPES, 50 μM beta mercaptoethanol, and 10% fetal calf serum).

B36

Please replace the paragraph beginning at page 116, line 6, with the following rewritten paragraph:

B37

For RNA preparation, probe labeling and Northern analysis protocols, see methods and procedures described in Example 2 above.

Please replace the paragraph beginning at page 116, line 28, with the following rewritten paragraph:

Clone (GI:7331559, GI: 9884693) has previously been mapped to the chromosomal location 1p31.1. The literature research reports that the mutations, deletions, rearrangements, disomies and/or breakpoints (in general: chromosomal aberrations) in below listed genes make the genes strong candidates for the onset of the listed diseases/disorders. Because the CLASP-3 gene is localized in the chromosome location 1p31.1, abnormal CLASP-3 gene regulation or deletion, rearrangement and/or mutations in CLASP-3 locus might be directly or indirectly associated with the onset of the listed diseases. Further, CLASP-3 gene can be used as a genetic probe to detect the abnormality in regions of these below listed genes and as a diagnostic marker for the related disease/disorders.

B38

Please replace the paragraph beginning at page 118, line 18, with the following rewritten paragraph:

BAC DNA was prepared from E. coli over night cultures using the QIAGEN DNA preparation system. All preps were performed according to the manufacturer's procedures, including the modifications for low copy number DNA constructs. Genomic DNA was prepared from HeLa cells (ATCC #CCL-17) using the methods described by Sambrook, Fritsch and Maniatis (1989); DNA concentrations were determined by the 260nm light absorption of the DNA solution, and aliquots corresponding to 20 microgram (μg) genomic DNA or 2 μg for BAC DNA were used for restriction enzyme digests with Eco RI or HindIII (genomic DNA) or Eco RI and Pst I (BAC DNA). Digests were carried out in 150 microliter volume for 4 hours at 37°C. Digested DNA was ethanol precipitated and the pellet was resuspended in 20 microliter

B39

B39 cont.

deionized water prior to migration over a 1.2 % agarose gel at 35 V over night. Running buffer was TAE, and the gel contained 0.1 μg ethidium bromide/ml to visualize DNA.

Please replace the paragraph beginning at page 119, line 28, with the following rewritten paragraph:

CLASP proteins are described in commonly assigned Application Nos. 09/736,969; 09/736,960; 09/736,968; (all filed December 13, 2000), 60/240,508, 60/240,503, 60/240,539, 60/240,543 (all filed October 13, 2000); 09/547,276, 60/196,267, 60/196,527, 60/196,528, 60/196,460 (all filed April 11, 2000); 60/182,296 (filed February 14, 2000), 60/176,195 (filed January 14, 2000), 60/170,453 (filed December 13, 1999), 60/162,498 (filed October 29, 1999), 60/160,860 filed October 21, 1999, 60/129,171 filed April 14, 1999, and in published PCT publications PCT/US00/13161 (WO 00/69896); PCT/US00/13205 (WO 00/69898); PCT/US00/13166 (WO 00/69897); PCT/US00/10158 (WO 00/61747); and PCT/US99/22996 (WO 00/20434). The disclosures of each of the aforementioned applications and publications is expressly incorporated herein by reference in its entirety for all purposes.

B40

IN THE CLAIMS:

Please cancel claim 1 without prejudice.

B41

1. An isolated Cadherin-like asymmetry protein-3 (CLASP-3) polynucleotide, wherein said polynucleotide is

(a) a polynucleotide that has the sequence of SEQ ID NO:1 or

(b) a polynucleotide that hybridizes under stringent hybridization conditions comprising wash conditions of 0.2X SSC and 0.1% SDS at 45°C to (a) and encodes a polypeptide having the sequence of SEQ ID NO:2 or an allelic variant or homologue of a polypeptide having the sequence of SEQ ID NO:2; or

(c) a polynucleotide that hybridizes under stringent hybridization conditions comprising wash conditions of 0.2X SSC and 0.1% SDS at 45°C to (a) and encodes a polypeptide with at 25 contiguous residues of the polypeptide of SEQ ID NO:2; or

B41 cancel.

(d) a polynucleotide that hybridizes under stringent hybridization conditions comprising wash conditions of 0.2X SSC and 0.1% SDS at 45°C to (a) and has at least 12 contiguous bases identical to or exactly complementary to SEQ ID NO:1.

B42 Such

4. An isolated Cadherin-like asymmetry protein-3 (CLASP-3) polynucleotide comprising a nucleotide sequence that has at least 90% percent identity to SEQ ID NO:1.

12. An antisense oligonucleotide complementary to a messenger RNA comprising SEQ ID NO:1 and encoding Cadherin-like asymmetry protein-3 (CLASP-3),

B43 wherein the oligonucleotide inhibits the expression of CLASP-3.

Such 8

13. An isolated DNA that encodes a Cadherin-like asymmetry protein-3 (CLASP-3) protein as shown in SEQ ID NO:2.

B44 Such 9

30. A pharmaceutical composition comprising a polynucleotide of claim 1 and a pharmaceutically acceptable carrier.